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PSA Prodrug-based multimodality agents for imaging metastatic prostate cancer.

PRINCIPAL INVESTIGATOR:
Ronnie C. Mease, Ph.D

CONTRACTING ORGANIZATION:
Johns Hopkins University
Baltimore MD 21218-2680

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Ronnie C. Mease

Email: rmease1@jhmi.edu

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14. ABSTRACT

The purpose of this work is to prepare and test a series of PSA binding prodrugs which contain both a fluorescent and radioactive group which can bind to enzymaticly active PSA in the vicinity of the tumor where the polar peptide portion is cleaved producing a lipophilic cleavage product which intercalates in the cell membrane of the tumor and nearly cells. In this period we made progress in the synthesis of iodinated and radioiodinated fluorescent PSA prodrugs and PSA cleavage products. In particular we were able to introduce the acid labile tributyl tin moiety needed to incorporate the radioiodine into the molecule towards the end of the synthetic route.

15. SUBJECT TERMS

Synthesis, PSA prodrugs, radionuclide and fluorescence imaging

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Our hypothesis is that a Prostate Cancer imaging agent can be developed using a prodrug approach where the prodrug is a polar radiolabeled ASP dye-PSA peptide which is selectively cleaved in the extracellular fluid of PCa to release an amphiphilic (charged and lipophilic) radiolabeled ASP dye which either intercalates into the plasma membrane of nearby cells or is internalized in cellular organelles of nearby cells. The Specific Aims for this project are as follows:

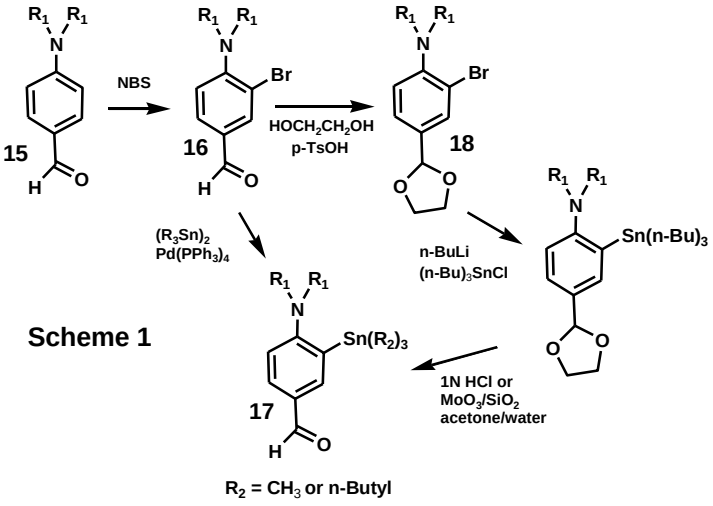
Aim 1. *Synthesis of a series of iodinated and radioiodinated fluorescent PSA Prodrugs and PSA cleavage products.* The drug group will consist of (Aminostyryl)pyridinium dyes (ASP dyes) with increasing carbon chain length and increasing number of fused benzene rings and will be conjugated to selected PSA substrate peptides to form the Prodrug.

Aim 2. *Identification of Prodrugs from Aim 1. which do not bind nonspecifically to cells in-vitro but whose PSA cleavage product does bind to cells, and determination of the site of localization (plasma membrane or internal organelle) of the cleavage product.*

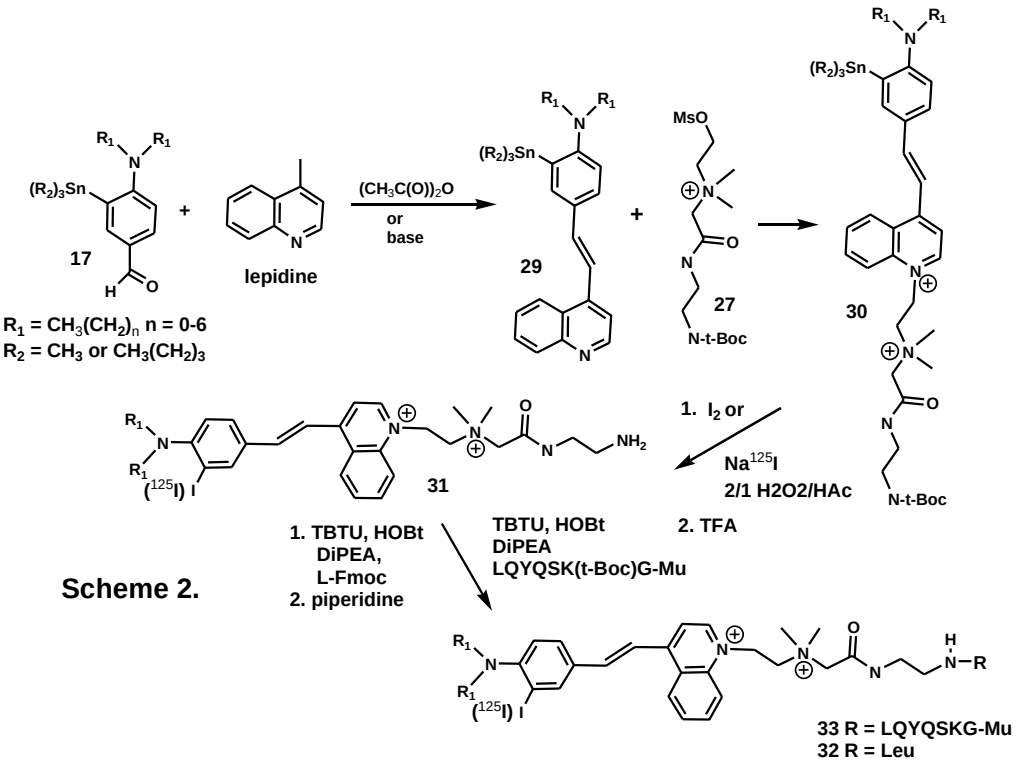
Aim 3. *Evaluation of promising Prodrugs from Aim 2 as agents for imaging Prostate Cancer in mice bearing PC-3 PSA+ and PC-3 PSA- tumor xenografts.*

Body:

In this period we have concentrated on Specific Aim 1. The synthesis of iodinated and radioiiodinated PSA Prodrugs and PSA cleavage products. The original proposed synthetic routes to these compounds are shown below in Schemes 1 and 2.

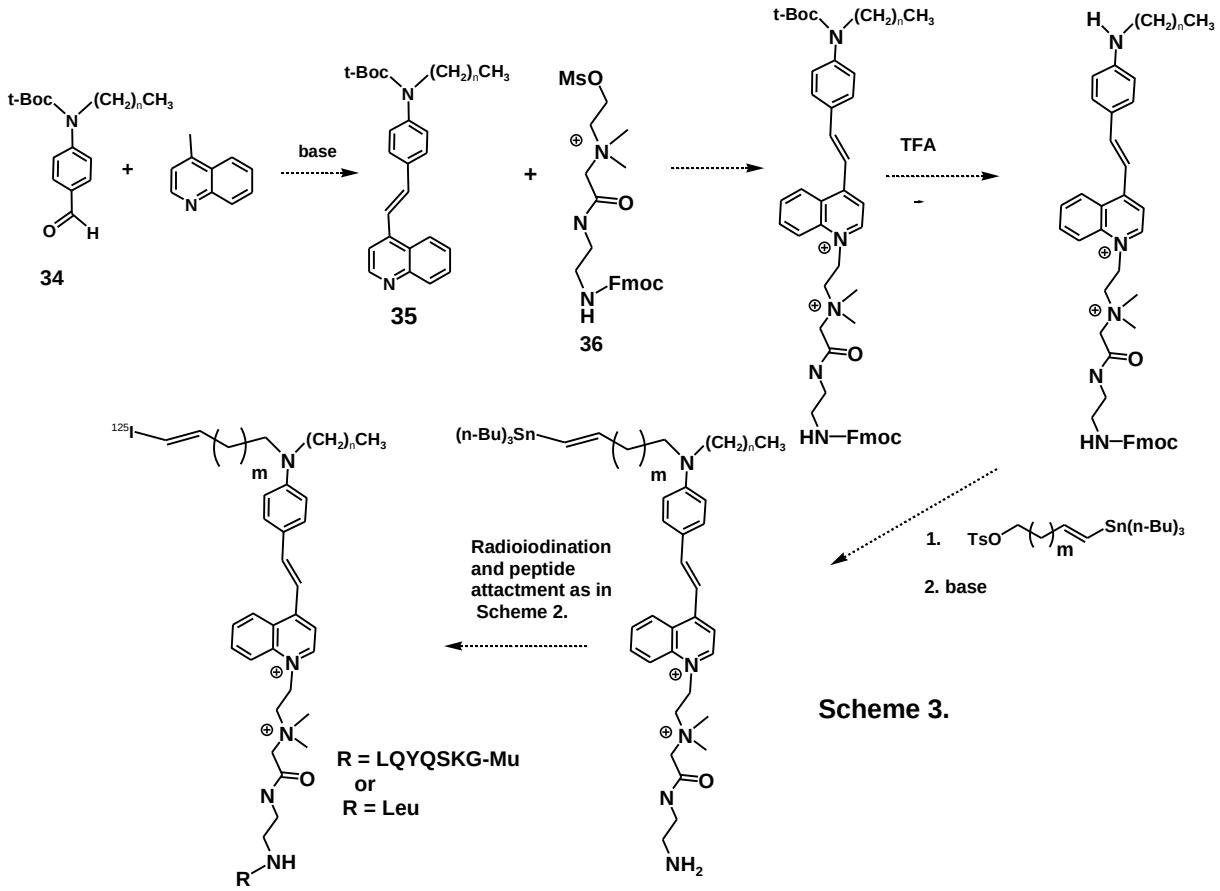


Scheme 1



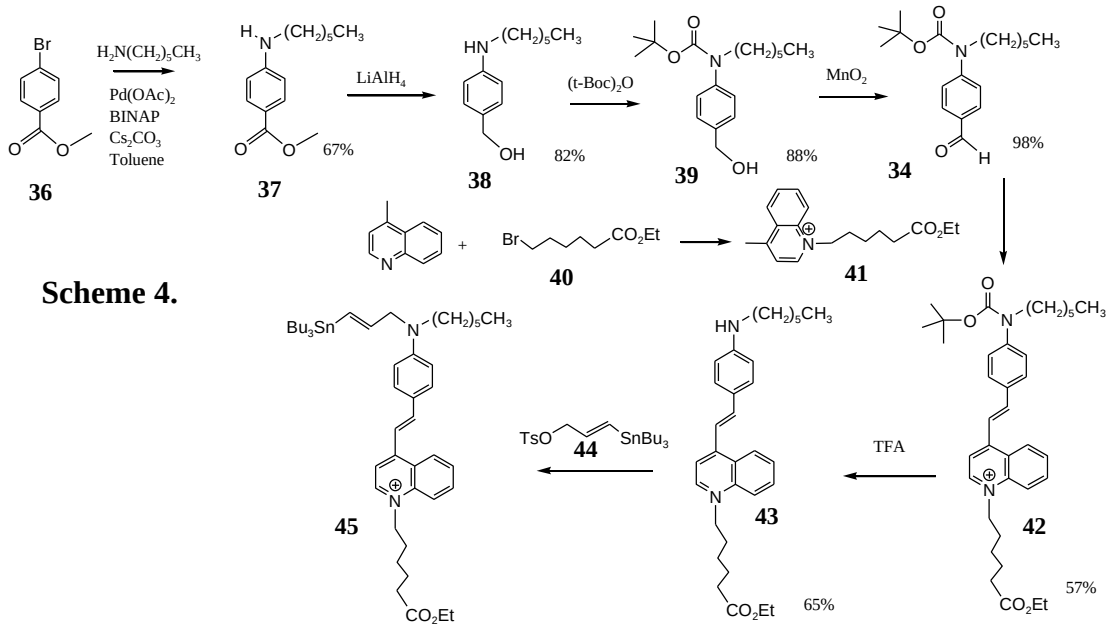
Scheme 2.

Notice that tributyl tin moiety which acts as the leaving group for the electrophilic iodination and radioiodination is formed early in the synthesis. Since organotin moieties are acid labile formation of this functional group early in the synthesis is probably not optimal. Therefore, we have been investigating a revised synthetic route where an alkyl vinyl tributyl tin moiety is attached to the monoalkylated aniline moiety late in the synthesis. A new proposed synthetic scheme is shown below in Scheme 3.



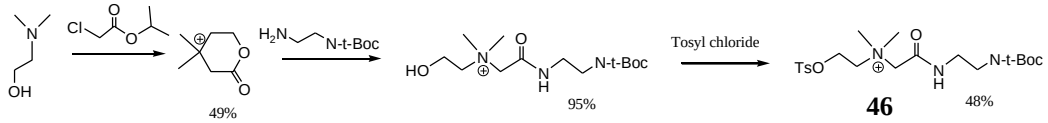
Scheme 3.

Progress on this route is discussed below and in Scheme 4. Commercial 4-bromo methyl benzoate compound **36** is monoalkylated to give **37** which is then reduced to alcohol **38**. The amino group of 38 is protected with t-Boc to give **39** which is then oxidized to give compound **34**. In our hands compound **34** does not readily condense with lepidine to give **35** (Scheme 3) but does condense with N-alkylated derivatives of lipidine. However, alkylation with tetraalkyl ammonium compounds like **27** and **36** has proven problematic and will be addressed further below. For investigation of this synthetic route lepidine was alkylated with 6-bromohexanoic acid ester **40** to give compound **41**. Compound **41** was then condensed with **34** to give compound **42**. Cleavage of the t-Boc group to give **43** and N-akylation with **44** gave crude compound **45**. This demonstrates that the tributyl tin moiety which is required for radioiodination can be added near the end of this synthetic route. Longer chain analogs of **44** can be prepared as described by Groh[1].



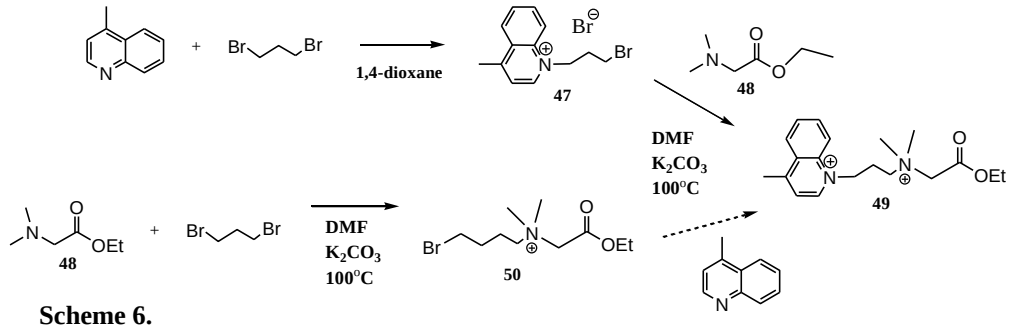
Scheme 4.

In our hands we have been unable to prepare compound **27** but have prepared related compound **46** as shown in Scheme 5 but **46** was unreactive with lepidine.



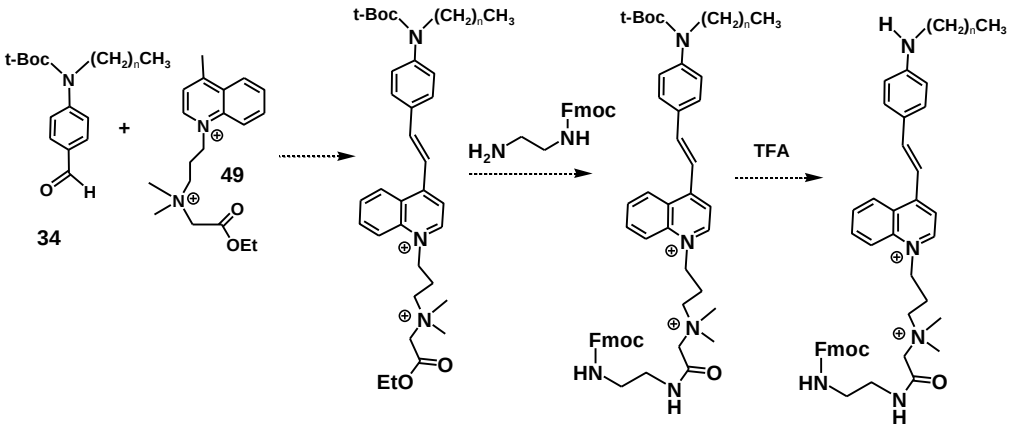
Scheme 5.

Therefore we have taken a stepwise route to the synthesis of an alkylated lepidine containing a tetraalkyl ammonium moiety within the linker. This approach is outlined in Scheme 6. Lepidine was alkylated with 1,3-dibromopropane to give compound **47**. This was then used to alkylate dimethylamino acetic acid ethyl ester compound **48** to give crude **49**. We are currently purifying this compound. We may also be able to prepare **49** by alkylating with compound **50** which was prepared by alkylating compound **48** with 1,3-dibromopropane.

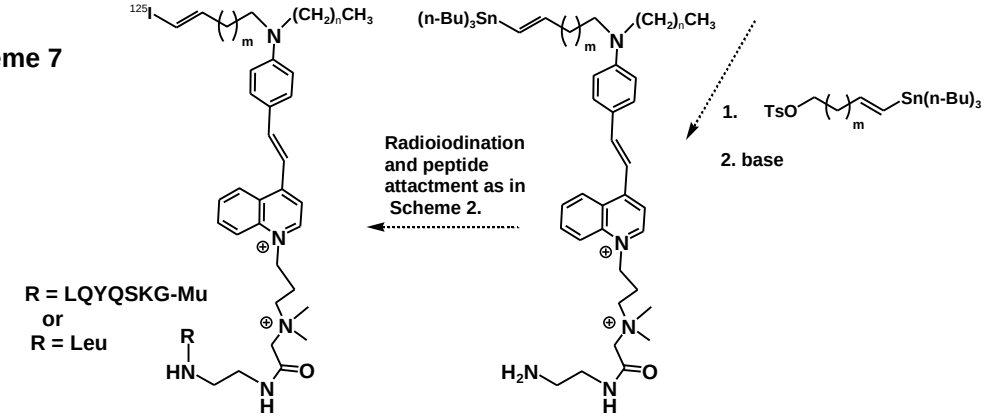


Scheme 6.

Once purified we envision using compound **49** in the synthesis of iodinated and radioiodinated fluorescent PSA Prodrugs and PSA cleavage products as outlined in Scheme 7.



Scheme 7



Key Research Accomplishments:

1. Developed a synthetic route to the desired targets where the acid labile tributyl tin moiety needed to incorporate the radioiodine into the molecule is introduced towards the end of the synthetic route.
2. Synthesized a key intermediate containing a tetra-alkylated ammonium moiety.

Reportable Outcomes:

1. **None**

Conclusions: We have made progress towards the synthesis of iodinated and radioiodinated fluorescent PSA Prodrugs and PSA cleavage products of Aims one and on which Aims two and three depend.

References:

1. Groh BL E-Vinylstannanes via stereospecific transmetallation with vinylalanes facilitates by lithium salts. Tetrahedron Letters 32(52) 7647-7650 (1991).

Appendices: None

Supporting Data: None